Elachistocleis bicolor (Amphibia: Anura: Microhylidae) and *Eupemphix nattereri* (Amphibia: Anura: Leuiperidae): Morphological abnormalities from Reserva Natural Laguna Blanca, Paraguay

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The global amphibian decline is well-documented, with the complex effects and interactions of ultraviolet radiation, predation, habitat modification, diseases, changes in climate or weather patterns, introduced predators, environmental acidity and toxicants all known to be causal factors (Alford and Richards, 1999). The drastic increase in the frequency of developmental abnormalities observed in recent years has also been linked to this phenomenon. Although the precise causes of such abnormalities are still imperfectly known, contamination from pollutants, viral and parasitic infections, ultraviolet radiation and predation in the early metamorphic stages, have all been mooted as potential contributors (Lannoo, 2008). Morphological abnormality of 0-5% is commonly found in amphibian populations, but when a population exceeds this mark it is considered abnormally high (Bionda *et al.,* 2012; Piha *et al.,* 2010; Pekkonen and Merilä, 2006).

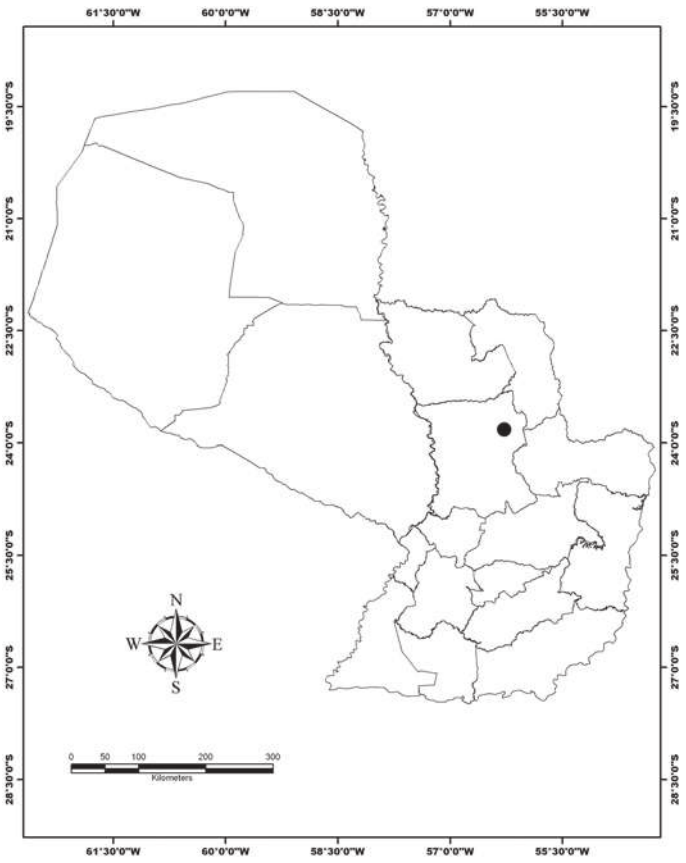


Figure 1: Map showing location of Reserva Natural Laguna Blanca, Departmento San Pedro, Paraguay.

Reserva Natural Laguna Blanca (RNLB) is located in the Cerrado zone of Departmento San Pedro, northeastern Paraguay (Fig. 1). It is a small, 804 ha reserve based around a freshwater lake of 157 ha (Guyra Paraguay, 2008). The reserve comprises a tract of 400 ha of near pristine Cerrado, a degraded section of Atlantic forest and a transitional zone of semi-deciduous, semi-humid gallery forest (Eiten, 1972; Eiten, 1978).

Specimens of *Elachistocleis bicolor* (Guérin-Méneville, 1838) (Microhylidae) and *Eupemphix nattereri* (Steindachner, 1863) (Lieuperidae) with malformations were collected near a temporary pool (23°46'52.6"S, 56°17'28.9"W) in a zone of transition between Cerrado and Atlantic Forest habitats at RNLB. The specimens are housed in the Colección Zoológica de Para La Tierra (CZPLT), located at the reserve. Morphological abnormalities were identified using Meteyer's (2000) classification. Comparative morphometry of the normal limb vs. abnormal limb of the two *E. bicolor* specimens and the two abnormal forelimbs of the *E. nattereri* specimen are given in tables 1 and 2. These specimens account for the first documented records of morphological abnormalities in amphibians from Paraguay.

Elachistocleis bicolor is a fossorial microhylid with a wide distribution comprising southern Brazil, Bolivia, Paraguay, Uruguay and northern Argentina (Weiler *et al.,* 2013). It can be found in various eco-regions including Atlantic Forest, Cerrado, Pantanal, and humid and dry Chaco (Caramaschi, 2010; Brusquetti and Lavilla, 2006; Nuñez *et al.,* 2004). New species of *Elachistocleis* are currently being described with regularity (Caramaschi, 2010; Kwet and Di-Bernardo, 1998; Lavilla *et al.,* 2003; Nunes de Almeida and Toledo, 2012; Pereyra *et al.,* 2013; Toledo, 2010; Toledo *et al.,* 2010), but specimens ascribed to *E. bicolor* here were those with a uniform yellow ventral surface, no dorsomedial line, and a head length less than 90% of the head width, in accordance with Caramaschi (2010).

Three specimens of *Elachistocleis bicolor* show abnormalities of the limbs (Table 1). CZPLT-H-487 (3 June 2013; female; 32.56 mm snout-vent length [SVL]; Fig. 2A-B) and

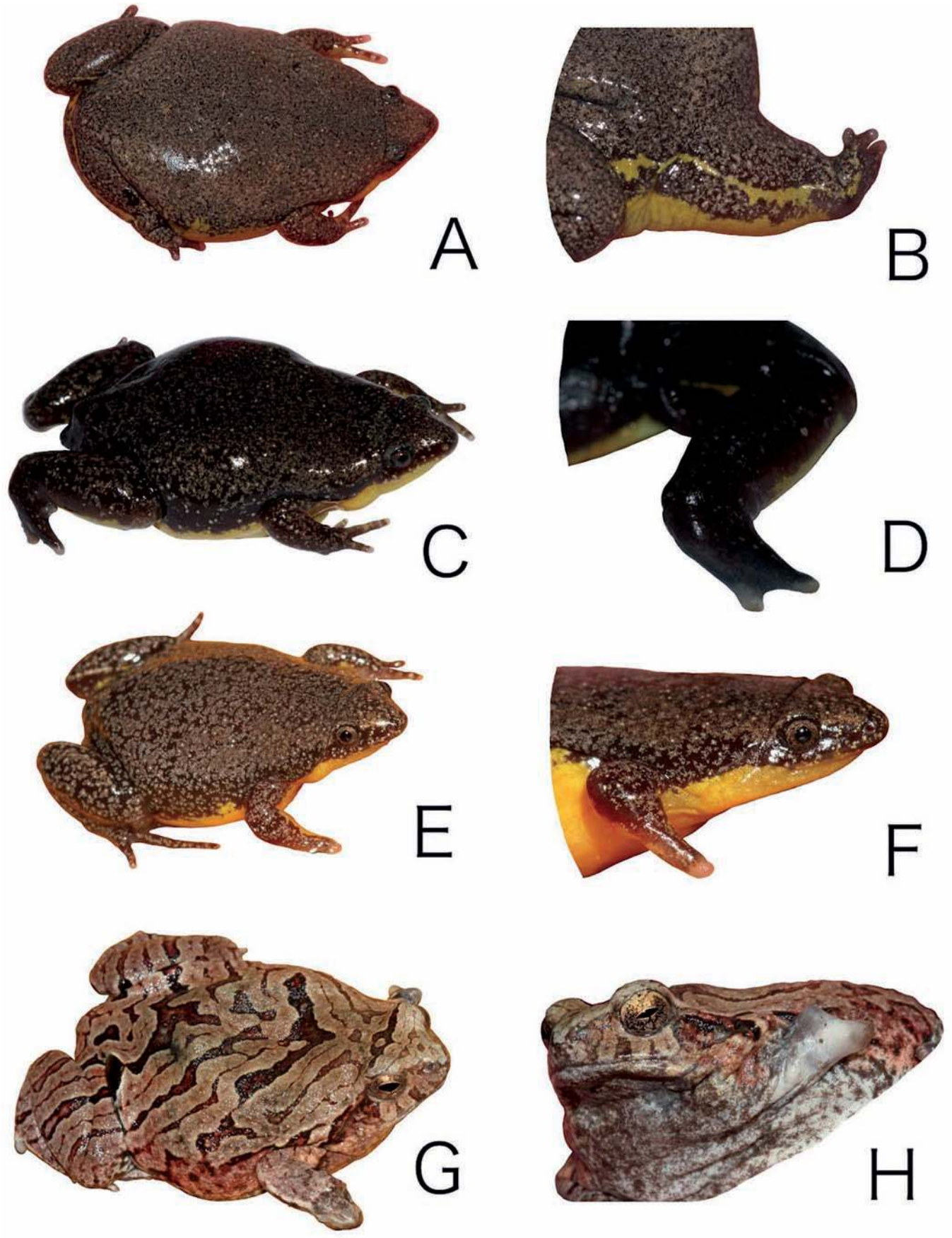


Figure 2: Morphological abnormalities described in the text from Reserva Natural Laguna Blanca, Departmento San Pedro, Paraguay. A-F: *Elachistocleis bicolor* specimens CZPLT-H-487 (A-B), CZPLT-H-521 (C-D) and CZPLT-H-584 (E-F). G-H: *Eupemphix nattereri* specimen CZPLT-H-586.

Table 1: Comparative morphometry of the normal limb vs. abnormal limb of *Elachistocleis bicolor* specimens. Measurements in mm.

	CZPLT-H-487 (abnormal)		CZPLT-H-521 (normal)	
	Left Side	**Right Side**	**Left Side**	**Right Side**
Femur	10.6	8.4	10.4	10.2
Tibiafibula	5.2	0	8.5	8.4
Foot	11.6	4.4	11.7	6.6

Table 2: Comparative morphometry of two abnormal forelimbs of the *Eupemphix nattereri* specimen 586. Measurements in mm.

	CZPLT-H-586	
	Left Side	**Right Side**
Humerus	12.0	12.1
Radio-ulna	3.1	10.6
Hand	0	4.2

CZPLT-H-521 (8 July 2013; male; 26.40 mm SVL; Fig. 2C-D) both have a reduced right hindlimb. CZPLT-H-487 displays hemimelia (reduction of the long bone) of the femur, an absent tibiafibula, ectrodactyly (absent digits) and brachydactyly (reduced digits) of the foot (three, reduced toes present compared to the norm of five), and rotation of the foot. CZPLT-H-521 shows ectrodactyly and brachydactyly. The femur, tibiafibula and tibiale/fibulare are present, but only two digits are complete. CZPLT-H-584 (29 November 2013; male; 23.04 mm SVL; Fig. 2E-F) has an abnormal right forelimb showing ectromelia of the tibiale/fibulare. The humerus and radio-ulna are complete, but the hand is reduced with no digits present.

Eupemphix nattereri is a widespread frog species distributed from central and southeast Brazil to eastern Paraguay and Bolivia (Weiler *et al.,* 2013). It can be found in a wide range of habitats including the humid Chaco, Cerrado, Atlantic Forest, and Pantanal (Brusquetti and Lavilla, 2006).

Specimen CZPLT-H-586 (29 November 2013; male; 47.7 mm SVL; Fig. 2G-H) has two abnormal forelimbs. The right limb shows ectromelia of the tibiale and fibulare (complete humerus and radio-ulna, but a reduced hand without digits), and the left limb shows ectromelia of the radio-ulna (complete humerus, reduced radio-ulna, and hand absent).

Despite ongoing year-round amphibian inventories at RNLB, morphological abnormalities have so far been discovered only on the limbs of these two species. Although the causes of the observed abnormalities are not immediately obvious, the low frequency of abnormalities and high level of species specificity, despite the large number of specimens and species collected, may be suggestive of a non-environmental cause, perhaps related to unsuccessful predation, accidental injuries or developmental errors (Guderyahn, 2006; Székely and Szilárd, 2003). All four specimens were adult and did not exhibit any obvious lack of fitness or detrimental health effects to suggest that such abnormalities might hinder their ability to survive.

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